

LC/MS/MS as a Potential Method for Characterizing Bacterial Contamination during Spacecraft Assembly

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Introduction

Exploration of outer bodies of interest to life's origins requires stringent measures to prevent contamination of these bodies with Earth life forms. Procedures are taken to vigorously clean spacecraft before launch. We are exploring MS to measure contamination during spacecraft assembly. MALDI and ESI-LC/MS create unique protein profiles from bacteria whole cells and cell lysates. Under ideal circumstances, these methods work well, but fail to yield consistent results on samples from different sources and handling techniques. Our more robust approach uses LC/MS/MS of the mixture of peptides obtained from the trypsin digestion of whole cell bacterial lysates

Methods

Bacillus subtilis vegetative cell (0.01% sodium azide) were aliquoted into tubes and the suspension was prepared for probe sonification by adjusting the concentration to 20% acetonitrile, 0.1% TFA (1). Cells were lysed on ice using a Branson sonifier 450 (20 pulses 5 times at 1 minute intervals). The lysate was centrifuged at 14,000 rpm for 30 minutes in the cold to remove cell debris then lyophilized. The proteins were denatured (7.2 M urea, 100mM NH₄HCO₃), reduced (4mM DTT) and alkylated (8mM iodoacetamide). After dialysis to remove the alkylating agent, the proteins were digested overnight by trypsin (2). The reaction was killed by the addition of 0.1% TFA. LC-MS-MS analysis was performed using a Finnigan LCQ, a custom built gradient loop HPLC and capillary (150u x 5cm) RP C18 column. All ion spectra were screened to eliminate poor MS/MS spectra using the winnow program prior to database search (3). Analysis by Sequest (4) was performed using an entire protein or nucleotide database and the output was screened for *Bacillus* hits. The output files were further analyzed re-screening through a *Bacillus* library (5). Only output with the same or higher correlation values were considered in the second screening.

Results

Previously our laboratory has used protein biomarker profiling as a method to identify bacteria (1). The method worked well for bacteria cultured from a single source. However, bacteria from different sources and prepared using different procedures did not yield reproducible results. In an effort to develop a more robust methodology, we have explored the use of trypsin to generate peptides that yield sequence data when analyzed by LC/MS. A trypsin digestion of whole cell lysate followed by LC/MS/MS analysis allowed the positive identification of specific *Bacillus subtilis* proteins. In an average sample run, the majority of the peptides identified with the highest correlation values belong to *Bacillus subtilis* proteins. These peptides represent 53% of total MS/MS spectra analyzed after filtering with winnow that cut out poor quality spectra.

We had originally hoped to create a library of commonly observed spectra to be used for bacteria identification however we found that the complexity of the cell lysate did not allow us to see the same peptide in every run. Methods to reduce the complexity of the mixture without sacrificing sensitivity may be of some benefit. It is important to point out that although the method is not reproducible it nevertheless does identify the organism. The detection of a few organism-specific peptides with high Xcorr values may be enough to check for contamination. When list of an observed peptide and their

detection from the same digestion mixture analyzed three times under the same set of conditions, we did not detect the same peptides in every run, in fact a diverse set is detected each time.

Although we did not intend to do a proteomics approach, we thought it would be interesting to look at a few of the proteins that were identified and their function. . Most of the proteins identified are expected to be present in high abundance in the cell. Proteins with the most number of hits included ribosomal proteins (50S ribosomal protein L7/L12 and 30S ribosomal protein S1 homolog), cold shock proteins (CSPB and CSPD), flagellin and a chaperone (trigger factor). The metabolic enzymes that were found in multiple runs include: malate dehydrogenase, alanine dehydrogenase, thioredoxin, triosephosphate isomerase, alkyl hydroperoxide reductase C22 protein, vegetative catalase and phosphocarrier protein HPR. We were also able to identify a few hypothetical proteins in the database such as conserved hypothetical protein yitW and hypothetical 39.7 KD protein in GLNQ-ANSR intergenic region. previously identified by open reading frames. We are unsure at this time whether the presence of these proteins have been detected by any other means. As more bacterial genomes are sequenced, this procedure can be applied to a variety of applications including the analysis of bacteria in distinct microenvironments

Conclusions

The described procedure depends on obtained fragment ion spectra of sufficient number of the most abundant and readily ionizable peptides present in the cytoplasm for that particular organism. For *Bacillus subtilis*, whose genome is in the database (7), we obtained specific peptide matches to expected proteins such as chaperonins, metabolic enzymes and ribosomal proteins. While the complex bacterial digestion mixture did not yield the same set of results each time different aliquots or concentrations were analyzed, the majority of high scoring matches were assigned to *Bacillus subtilis* proteins. We consistently matched a sufficient number of *Bacillus subtilis* proteins to be certain we were identifying the organism. Because the method is simple and does not require any special skills or instrumentation, it can be easily reproduced with a variety of applications. Factors such as where the organism grows, what it eats, whether it is present as spore or vegetative cell, while may affect what proteins are detected will not affect the ability to detect the organism.

Acknowledgements

Curtis Croker for constant modification of our software-controlled HPLC and the peak parking procedure which will be used for any further separations. John Percy for his programming skills used for the creation of the MoWeD deconvolution software which was initially used in the protein profiling experiments. Roger Moore for creation of the winnow program which was used as an effective screen for MS/MS spectra.

References

1. Krishnamurthy et al (1999) "Liquid chromatography/microspray mass spectrometry for bacterial investigations" *Rapid Commun Mass Spectrom* 13(1):39-49
2. Lee and Shively (1990) "Enzymatic and Chemical Digestion of Proteins for Mass Spectrometry" *Methods in Enzymology* 193:361-374.
3. Moore et al. (2000) "Method for Screening Peptide Fragment Ion Mass Spectra Prior to Database Searching" *JASMS* 11:422-426
4. Yates et al. (1998) " Method to compare collision-induced dissociation spectra of peptides: potential for library searching and subtractive analysis" *Anal Chem* 70:3557-65
5. National Center for Biotechnology Information (NCBI) biomedical databases
6. Altschul et al (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402.
7. Kunst et al (1997) "The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*" *Nature* 390:249